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Yang, M

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Complete List of Authors:	Yang, Meng; China Unversity of Geoscience Beijing Zhong, Yuezhi; China Unversity of Geoscience Beijing Zhang, Baogang; China Unversity of Geoscience Beijing, Shi, Jiaxin; China Unversity of Geoscience Beijing Huang, Xueyang; China Unversity of Geoscience Beijing Xing, Yi; University of Science and Technology Beijing Su, Lin; Southeast University Liu, Huipeng; China Unversity of Geoscience Beijing Borthwick, Alistair G.L.; University of Edinburgh School of GeoSciences
Keywords:	Sulfide, Nano-sheets, Microbial fuel cells, Microbial community, Carbon-fiber-felt

Enhanced sulfide removal and bioelectricity generation in microbial fuel cells with anodes modified by vertically oriented nanosheets

Meng Yang[a,#], Yuezhi Zhong[a,#], Baogang Zhang[a,*], Jiaxin Shi[a], Xueyang Huang[a], Yi Xing[b,*], Lin Su[c], Huipeng Liu[a], Alistair G.L. Borthwick[d]

[a] *School of Water Resources and Environment, MOE Key Laboratory of Groundwater Circulation and Environmental Evolution, China University of Geosciences (Beijing), Beijing 100083, P. R. China*

[b] *School of Energy and Environmental Engineering, University of Sciences and Technology Beijing, Beijing Key Laboratory of Resource-oriented Treatment of Industrial Pollutants, Beijing 100083, P. R. China*

[c] *State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096, P. R. China*

[d] *School of Engineering, The University of Edinburgh, The King's Buildings, Edinburgh EH9 3JL, UK*

Abstract

Anode materials and structures are of critical importance for microbial fuel cells (MFCs) recovering energy from toxic substrates. Carbon-fiber-felt anodes modified by layers of vertically oriented TiO_2 and Fe_2O_3 nanosheets respectively were applied in present study. Enhanced sulfide removal efficiencies (both over 90%) were obtained after 48 h operation, with maximum power densities improved by 1.53 and 1.36 folds compared with MFCs with raw carbon-fiber-felt anode, respectively. The

[#] These authors contributed equally to this work.

[*] Corresponding author. Tel.: +86 10 8232 2281; fax: +86 10 8232 1081.

E-mail: zbgcugb@gmail.com, baogangzhang@cugb.edu.cn (B. Zhang); xing_bkd@163.com (Y. Xing).

modified anodes provided more active sites for microbial adhesion with increasing biomass densities. High-throughput 16S rRNA gene sequencing analysis also indicated the increase of microbial diversities. Bacteroidetes responsible for bioelectricity generation with *Thiobacillus* and *Spirochaeta* dominating sulfide removal were found in the MFCs with the modified anodes, with less anaerobic fermentative bacteria as Firmicutes appeared. This indicates that the proposed materials are competitive for applications of MFCs generating bioelectricity from toxic sulfide.

Keywords: Sulfide; Nano-sheets; Microbial fuel cells; Microbial community; Carbon-fiber-felt

1. Introduction

Sulfide is a hazardous, corrosive and odorous substance that often occurs in industrial wastewaters, especially in effluent from viscose rayon industries, petrochemical plants and tanneries [1,2]. Sulfide is toxic to human health, with studies showing that sulfide is particularly harmful to cytochrome c oxidase and causes cell hypoxia [3]. Thus, sulfide-contaminated wastewater must be treated thoroughly before discharge into the environment. Although common physical-chemical methods, such as adsorption and chemical oxidation, can remove sulfide, they are costly to implement and require high energy inputs [4]. In contrast, biological processes provide an environmental-friendly alternative for sulfide removal from both liquids and gases under ambient environmental conditions [5-7].

Microbial fuel cells (MFCs) are nowadays recognized to be renewable, clean energy sources that can generate bioelectricity during wastewater treatment [8,9]. Numerous pollutants have been successfully handled with energy recovery based on MFC technology, including organics and metals [10-12]. Additionally, anode materials and structures are particularly important regarding the performance of MFCs in removal of toxic substances: properly designed anodes can help electricigens to collect electrons from anolyte and thus sustain bioelectricity generation while tolerating harmful substrates [13]. Carbon-based anodes are most frequently used [14] and further gains in performance arise from coating them with conducting polymers or metal oxides [15,16]. Nowadays, hydrothermally synthesized layers of vertically oriented metal oxides nanosheets such as TiO_2 and Fe_2O_3 with activated carbon (AC) powder in situ on the surface of carbon paper exhibit excellent behavior as MFCs' anodes as they have high biocompatibility, support mass transport, have large surface area for adhesion of bacteria, and provide direct pathways for electrons movement to the electrode [17,18]. Furthermore, carbon fiber felt is more promising as anodic material for scale-up of MFCs because it offers a more suitable support for bacteria attachment and growth, higher mechanical strength, more active sites for the chemical reactions [19-21], while its performances after being modified by layers of vertically oriented metal oxides nanosheets are rarely reported. Studies have been carried out on sulfide removals using MFCs [4,22,23]. In anode chambers, sulfide acts as electron donor and it is biochemically oxidized based on Equ. (1) and (2), with anodic electrodes as electron acceptors:

$$HS^- \rightarrow S^0 + H^+ + 2e^- \quad (1)$$

$$S^0 + 4H_2O \rightarrow SO_4^{2-} + 8H^+ + 6e^- \quad (2)$$

Commonly, co-substrate such as glucose is supplied as co-existing electron donor [4], which is oxidized as Equ. (3),

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad (3)$$

Moreover, other contaminants with higher redox potentials such as nitrate may be presented with sulfide [22]. It can also act as competitive electron acceptor, which is reduced as Equ. (4),

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \quad (4)$$

Then electrons flow to cathode via external circuits. In cathode chambers, oxygen and potassium ferricyanide are most frequently used as electron acceptors and they are reduced as Equ. (5) and (6) [23,24],

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (5)$$

$$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-} \quad (6)$$

While most of these studies focus on co-existing pollutants removals [4,22,24-26], operating factors investigations [27] and commercial electrodes comparisons [28]. Little attention has been paid to date on modified carbon-based anode especially for carbon fiber felt to enhance bioelectricity generation and sulfide removal in the context of accumulation of microbes in anode chambers with toxic matrix.

The present research explores carbon-fiber-felt anodes modified with two kinds of metal oxides (TiO_2 and Fe_2O_3) nanosheets respectively that are vertically oriented on the surface. Enhanced performances in aspects of power outputs and sulfide removals

are investigated for MFCs equipped with these anodes, compared with unmodified one. The amounts and species of accumulated microbes are also analyzed. This work provides a potential alternative for treating sulfide-contaminated wastewater by MFC technology with proposed anodes.

2. Materials and methods

2.1 Preparation of anodes and fabrication of MFCs

TiO_2 and Fe_2O_3 sols were synthesized in the laboratory following a previous report [17,18]. Clean carbon-fiber-felt materials (3 mm thickness, 4 cm length and width, provided by Beijing Evergrow Resources CO., LTD, China) were immersed in the TiO_2 and Fe_2O_3 sols for 10 min and dried at 80 °C. After that, the materials were calcined for 30 min in a tubular furnace at 350 °C so that a TiO_2 and Fe_2O_3 seed layers formed on the surfaces of the carbon-fiber felts. A Teflon-lined stainless steel autoclave (50 mL in volume) filled with 40 mL of aqueous solution of 10 M NaOH and 0.2 g of AC powder was placed in an oven at 180 °C for 24 h. After the carbon-fiber-felts had cooled down to room temperature, the modified carbon-fiber felts were rinsed with ultrapure water to remove AC, followed by soaking with 0.1 M hydrochloric acid for 1 h, then washed to neutral with deionized water and dried at 80 °C. Sequentially, the samples were calcined at 550 °C for 1 h in a N_2 atmosphere [17,18].

Three H-type MFCs with cylindrical chambers (250 mL for each) were fabricated as previously described [29]. The two chambers for each MFC were divided by a

proton exchange membrane (Nafion117#. Dupont, USA) with a surface area about 4 cm^2. The MFCs were equipped with TiO_2 nanosheets modified carbon-fiber-felt anode (MFC-TiO_2) and Fe_2O_3 nanosheets modified carbon-fiber-felt anode (MFC-Fe_2O_3), respectively, as prepared above, with surface area of 16 cm^2. Raw carbon-fiber-felt anode without modification was also employed as control (MFC-CF). All cathodes were made of ordinary carbon-fiber-felt with the same size as anodes. The anode and cathode were connected across a 100 Ω external resistor. Each anode chamber was inoculated with 25 mL anaerobic granular sludge from an up-flow anaerobic sludge blanket (UASB) reactor treating high strength sulfate wastewater. The anolyte included the following (per L): 0.75 g of $C_6H_{12}O_6$; 5.62 g of $NaH_2PO_4 \cdot 2H_2O$; 6.15 g of $Na_2HPO_4 \cdot 12H_2O$; 0.31 g of NH_4Cl; 0.13 g of KCl; 1.25 mL of vitamin solution; and 12.5 mL of trace mineral element solution. Sulfide was added to the anode solution in the form of $Na_2S \cdot 9H_2O$ with concentration of 60 mg L^{-1} to facilitate the comparison [30]. The catholyte included the following (Per L): 9 g of KH_2PO_4, 8 g of $K_2HPO_4 \cdot 3H_2O$ and 0.05 g $K_3[Fe(CN)_6]$.

2.2. Experimental procedures

Morphology and composition of the modified anodes were analyzed first. Then successful start-up of all MFCs was realized by refreshing anolyte every 2 days. After that, the power outputs and sulfide removals in a typical cycle (48 h) were evaluated, noting that most of the sulfide was removed within that time. Electrochemical measurements were also undertaken for the three types of MFC. After 3 months

operation with refreshment of anolyte every 2 days, ultrasonic was employed to collect the bacteria attached to the surfaces of the anodes, and the samples then used for high-throughput 16S rRNA gene sequencing on MiSeq (Illumina, the USA). All experiments were carried out at room temperature (22 ± 2 ℃) for practical application consideration and easy comparison with existing studies [22]. For each condition, trials were carried out in triplicate and average results from the three MFCs were reported.

2.3. Analytical methods and data representation

Measurement of chemical oxygen demand (COD) was undertaken based on digestion with potassium dichromate in concentrated sulfuric acid for 2 h at 150 ℃. Sulfide was determined according to the methylene blue method (n = 665 nm) [31]. The indication of ‘‘sulfide’’ described all species (H_2S, HS^-, and S^{2-}). Total organic carbon (TOC) was monitored by Multi N/C 3000 TOC analyzer (Analytik Jena AG, Germany). Sulfate was measured by ion chromatography (ICS-1600, Dionex, the USA). pH was measured using a pH-201 meter (Hanna, Italy). The biomass on the anode surface was determined using the phospholipid analysis as previously described and the biomass density was expressed as the mass of phosphorus normalized by anode volume (48 cm^3) [32]. The morphology and composition of modified anodes were analyzed by a JEOL JAX-840 scanning electron microscope (SEM) operating at 20 kV with an energy dispersive X-ray (EDX).

Voltage measurements were taken using a voltmeter throughout the test. The

polarization curve and power outputs were obtained by varying the external resistance over the range from 10 to 5000 Ω. For each point on the polarization curve, readings were taken when pseudo-steady-state conditions was established, which might take several minutes or more [4,33,34]. MFCs operated at least twice under each resistance to ensure the repeatability of power outputs. Power density was normalized to the single-side projected surface area of the anode. Cyclic voltammetry (CV) measurement was carried out at a scan rate of 2 mV s^{-1} in the range of -1 V to +1 V using an electrochemical workstation (VMP3, Bio-Logic Science Instruments, France) with Ag/AgCl as reference electrode. Electrochemical impedance spectroscopy (EIS) measurement was conducted over a frequency range of 100 kHz to 1 mHz with an AC signal of 10 mV amplitude. All electrolyte solutions were deaerated by high-purity nitrogen for at least 15 minutes prior to the measurement [32]. Coulombic efficiency (CE) was calculated as reported previously [4].

Molecular biology analysis was performed to obtain the characteristics of the microbial population. Total genomic DNA was collected, pooled, and amplified according to previous procedures [35]. Then a mixture of amplicons was used for high-throughput 16S rRNA gene sequencing on MiSeq (Illumina, the USA). Raw sequencing data were deposited in the NCBI Sequence Read Archive with access number of SPR067096 and were analyzed according to Hao et al. [35].

3. Results and discussion

3.1 Characteristics of the modified anodes

It was observed that the modified carbon-fiber-felt surfaces were covered with dense layers, comprising vertically oriented nanosheets on top, which formed vertical pores through the layer, compared with raw carbon-fiber-felt anode (Fig. 1a, Fig. 1b, Fig. 1c). This structure improved the roughness of the anode surface, which was similar with previous studies [17,18] and meant that the 3D open porous structure was favorable to biofilm growth as well as permeability of electrolyte, substrates, and electron mediators [36]. These vertically oriented nanosheets extending upward from the surface of carbon-fiber-felts could provide direct pathways for electrons to transfer from exoelectrogens in the biofilm to the carbon-fiber-felts. Moreover, carbon-fiber-felts with larger specific surface areas were selected as the basic material in present study instead of plain carbon paper, which would provide more sites for microbes' attachment [17,18]. The compositions of the modified anodes were examined using EDX and the corresponding elements (Ti, Fe, O) were detected (Fig. 1d), indicating that the anodes were well modified with metal oxides. It was interesting to note that some metal oxides could not only enhance the interfacial electron transfers in MFCs [17] but also stimulate the growth of chemoautotrophic and heterotrophic bacteria using solar energy [37]. This implied that the proposed modified anodes were promising and ready for following experiments.

3.2 Bioelectricity generation by the MFCs

Polarization curves were obtained using closed-circuit MFCs during 48 h operation (Fig. 2a). MFC-TiO_2 exhibited the highest maximum power density of 607.8 ± 16.1 mW m^{-2} at current density of 1591.38 mA m^{-2} with 150 Ω external resistances, followed by MFC-Fe_2O_3 with this value of 537.6 ± 14.8 mW m^{-2} at current density of 1296.15 mA m^{-2} with 200 Ω external resistances, 1.53 and 1.36 folds higher than the maximum power output of MFC-CF (396.1 ± 11.7 mW m^{-2} with 400 Ω external resistances), while the maximum power output of MFC-CF was slightly higher than that obtained from previous study, where maximum power output of 283 mW m^{-2} was obtained in dual chamber MFCs (300 mL net volume for anode chamber) with 300 mg L^{-1} initial sulfide concentration [25]. The results indicated that the modified anodes with the TiO_2 and Fe_2O_3 nanosheets vertically oriented on the surface of carbon-fiber-felt could increase the power output of MFCs by providing more sites for microbes' attachment and more direct electrons transfer pathways [17,18].

Anode potentials which dominated the above difference were also monitored during this period (Fig. 2b). The anode potentials of the MFC-CF were lower than those obtained with sulfide free substrate due to lower redox potential of sulfide [38,39]. Additionally, the sulfide added to the anodic solution and the sulfate produced through sulfide oxidation acted as a soluble redox mediator, which could promote electron transfer from the bacterial cells to the anode surface [40]. The anode potentials of both MFC-TiO_2 and MFC-Fe_2O_3 were further lower than those of MFC-CF. It should be noted that previous results had shown that the lower negative

anode potential could demonstrate better activity of anode communities [41]. Moreover, metal oxides possess superior electronic, optical, and dielectric properties and had been employed for surface modifications of anodes in MFCs for high power output [36]. Hence, the present results indicated that carbon-fiber-felt with TiO_2 and Fe_2O_3 nanosheets vertically oriented on the surface as anode materials could facilitate adhesion, growth and activity of bacteria for enhancement of MFC performance.

The electrochemical behaviors of the modified anodes were also characterized. More obvious redox peaks and much higher redox peak currents than untreated carbon-fiber-felt anode were observed in CV curves, especially with TiO_2 nanosheets (Fig. 3a). This indicated that electrodes with metal oxides nanosheets vertically oriented on the surface of carbon-fiber-felt had better electron transfer properties than an untreated electrode [42]. EIS was used to characterize the electrode surface and to evaluate the kinetics of the electrochemical reaction and dramatically decrease of internal resistances for MFCs with modified anodes were observed in Fig. 3b. Charge transfer resistances derived from the MFC-TiO_2 and MFC-Fe_2O_3 were also remarkably smaller than that of the untreated electrode, consistent with the CV behavior and showing the advantage of the proposed modification in electron transfers.

3.3 Sulfide and organics removals in the MFCs

Concentrations of sulfide and generated sulfate as well as TOC were monitored initially and after 48 h operation (Fig. 4). Both sulfide and TOC declined after the operation, demonstrating the feasibility of MFC technology for the removal of sulfide

and organic carbon simultaneously. Similar as the power outputs, sulfide removal efficiencies of MFC-TiO_2 (94.4 ± 1.5%) and MFC-Fe_2O_3 (91.6 ± 1.7%) were higher than that of MFC-CF (88.5 ± 1.9%), with the removal efficiency obtained from MFC-CF comparable with results from dual chamber MFCs that 84.7% of 100 mg L^{-1} sulfide in the influent was removed within 72 h [4] and higher than removal efficiency of 60% with 80 mg L^{-1} initial sulfide concentration in 72 h operation [26]. Nevertheless, sulfide removal efficiencies obtained in this study were relatively lower than those achieved by Cai et al. [43], where above 99% of added sulfide were removed, as our used cathode electron acceptor ($K_3[Fe(CN)_6]$) possesses lower redox potential than their employed $KMnO_4$ [24]. The TOC removal efficiencies also exhibited the similar principles, with MFC-TiO_2 (56.9 ± 1.8%) and MFC-Fe_2O_3 (55.2 ± 1.4%) higher than that of MFC-CF (31.7 ± 1.7%). The removal efficiencies of sulfide were superior to the TOC indicated that sulfide and organics acted as co-electron donors with competitive relationship and sulfide was easier to be oxidized than organic matter because of its lower redox potential [44,45]. Moreover, the calculated CE based on COD of the MFC-TiO_2 (13.2 ± 1.7%), MFC-Fe_2O_3 (12.2 ± 1.6%) and MFC-CF (11.4 ± 1.8%) was comparable with similar systems as previously reported [4]. This implied that carbon-fiber-felt with these two kinds of metal oxide (TiO_2 and Fe_2O_3) nanosheets vertically oriented on its surface as promising anode material could enhance both sulfide and organics removals in MFCs.

Oxidation products of sulfide in the MFCs were investigated. After 48 h operation, concentrations of generated sulfate in the exhausted anolyte were 3.26 ±

0.97 mg L^{-1} in MFC-TiO_2 and 2.74 ± 0.89 mg L^{-1} in MFC-Fe_2O_3, respectively (Fig. 4), with other soluble species ($S_2O_3^-$, SO_3^{2-}) undetected in all MFCs, suggesting that most oxidation products were insoluble. In fact, many obvious solid particles covered on the anode surfaces after operation (Figure S1, Supporting Information). The particles were examined using EDX and it was found that the main component was elemental sulfur (Fig. 1d), which was the expected oxidation product as it was a non-corrosive solid and was easy to remove from aqueous solutions [4]. The present results suggested that elemental sulfur was the main product for sulfide removal when the electrochemical and biological oxidations were performed in MFCs, also proved by previous studies [4,46]. Recovery and quantification of generated elemental sulfurs were difficult as they mixed with biofilms. The relatively lower sulfate concentration and higher peak of elemental sulfur indicated that the modified carbon-fiber-felt electrodes were more amenable to biofilm growth, enabling more sulfur to be generated.

3.4 Identification of the involved microbes

The monitored voltage outputs and sulfide removals were relatively stable during the whole experiment. After 3 months operation, it was found that the biomass densities of the MFC-TiO_2 (26.7 μg cm^{-1}) and MFC-Fe_2O_3 (22.1 μg cm^{-1}) were higher than that of MFC-CF (16.2 μg cm^{-1}), suggesting more microbes adhering on the modified anodes (Figure S1, Supporting Information). Total numbers of operational taxonomy units (OTUs) estimated by Chao and Ace estimators with infinite sampling in MFC-TiO_2 and MFC-Fe_2O_3 were much larger than that in MFC-CF (Table 1),

indicating that MFC-TiO_2 and MFC-Fe_2O_3 possessed greater richness of microbial diversity than MFC-CF. Both Simpson and Shannon diversity index provide not only the simply species richness (i.e., the number of species present) but how the abundance of each species is distributed (the evenness of the species) among all the species in the community. The increase of Shannon index and decrease of Simpson index of MFCs with modified anodes compared with raw one implied the bacterial communities in MFC-TiO_2 and MFC-Fe_2O_3 were more diverse than those in the MFC-CF due to the stimulated growth bacteria with the added metal oxides [36]. Besides, visible light-excited photoelectrons from metal oxide could stimulate the growth of chemoautotrophic and heterotrophic bacteria [37].

16S rRNA gene sequence and taxonomy analyses for the microbes in the three MFCs were performed at phylum, class and genus levels in order to understand the role of bacteria in enhanced sulfide oxidation and bioelectricity generation (Table 2). Electrochemically activated bacteria that were conducive to bioelectricity generation were enriched in the MFCs, especially with modified anodes. Bacteroidetes, the most frequently appeared species in the anode biofilms of MFCs with electrochemical activity as reported by Ha et al. [46] were enriched in MFC-TiO_2 (4.94%) and MFC-Fe_2O_3 (1.45%) than those in MFC-CF (1.22%). Moreover, with the enhancement of MFCs' functions of electricity generation and sulfide removal, plenty of bacteria with electrochemical activity were domesticated, such as the Deltaproteobacteria species, especially in the MFCs with modified anode. This implied that the design with TiO_2 and Fe_2O_3 nanosheets vertically oriented on the

surface of carbon-fiber-felt could accumulate more electrochemically activated bacteria to generate bioelectricity than the untreated anode.

Sulfur related bacteria responsible for sulfide oxidation and sulfate reduction were also detected. *Thiobacillus* in Alphaproteobacteria, a famous sulfur-oxidizing bacterium that can oxidize sulfur to sulfate was enriched in MFC-TiO_2 [43,47]. *Spirochaeta* of Spirochaetes requiring sulfide in the growth medium and oxidizing it non-enzymically to elemental sulfur was found in MFC-Fe_2O_3 [48]. Sulfate-reducing bacteria were also greatly enhanced in the MFCs with the proposed anodes, such as *Desulfovibrio* genus of Deltaproteobacteria, which could reduce sulfate as well as other sulfur species such as sulfite and thiosulfate [4,23]. These species worked together to realized higher sulfide removals in the improved MFCs. *Pseudoxanthomonas* of Gammaproteobacteria with an abundance of 4.63% in MFC-CF could be responsible for sulfide removals in this reactor as this species can oxidize sulfide to sulfate [49]. More species of sulfur related bacteria were found in the three MFCs than previously reported by Sun et al. [50] who employed sulfide as the sole electron donor in the MFCs; the greater variety and numbers of bacteria probably occurred due to the complex substrate (glucose and sulfide) employed in the present study.

Less anaerobic fermentative bacteria without electrochemical activity appeared in the MFCs with modified anodes. Firmicutes accounted for the largest portion of bacteria found in anaerobic sludge [51] decreased more greatly in MFC-TiO_2 and MFC-Fe_2O_3 than in MFC-CF. *Rhodobacter* sp. (belonging to Alphaproteobacteria)

promoting anaerobic fermentation [52] also exhibited the similar principles. These indicated that anaerobic fermentation process competing with bioelectricity generation was further weakened when MFCs equipped with carbon-fiber-felt anodes with TiO_2 and Fe_2O_3 nanosheets vertically oriented on their surfaces.

4. Conclusions

The surfaces of carbon-fiber-felt were successfully modified by layers of vertically oriented TiO_2 and Fe_2O_3 nanosheets respectively and acted as anodes in MFCs. Higher maximum power outputs of MFC-TiO_2 (607.75 mW m^{-2}) and MFC-Fe_2O_3 (537.63 mW m^{-2}) were obtained, compared with the MFC with untreated carbon-fiber-felt (396.05 mW m^{-2}), and the sulfide and TOC removal efficiencies also increased. These results could be contributed to more active sites for microbial adhesion with increasing biomass densities by the modified anodes. Increases of microbial diversities were also observed by high-throughput 16S rRNA gene sequencing analysis and specific functional species were found, such as the enhanced Bacteroidetes responsible for bioelectricity generation with *Thiobacillus* and *Spirochaeta* dominating sulfide removal, with less anaerobic fermentative bacteria Firmicutes.

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Figure Captions.

Fig. 1. SEM images and EDX spectral intensities. (a) with TiO_2 nanosheets, (b) with Fe_2O_3 nanosheets, (c) bare carbon-fiber-felt, (d) corresponding EDX spectral intensities.

Fig. 2. (a) Polarization curves and power outputs as well as (b) anode potentials of MFCs with three kinds of anodes.

Fig. 3. (a) CV and (b) Nyquist plot of EIS data for the anodes of three MFCs.

Fig. 4. Changes of sulfide and TOC during 48 h operation as well as the generated sulfate in the anode chambers of three MFCs. The black legend referred to left vertical axis while the red legend referred to the right vertical axis.

512 **Table 1.** Alpha-diversity of three MFCs employed in this study.

Type	Ace	Chao	Shannon	Simpson	Coverage
MFC-TiO_2	768	760	4.33	0.031	0.996
MFC-Fe_2O_3	505	493	2.69	0.192	0.996
MFC-CF	394	375	2.45	0.240	0.995

Table 2. Percentages of sequences identified to different phylogenies in the MFCs with three different anodes.

Phylum	Class	Genus	MFC-TiO_2 (%)	MFC-Fe_2O_3 (%)	MFC-CF (%)	Phylum	Class	Genus	MFC-TiO_2 (%)	MFC-Fe_2O_3 (%)	MFC-CF (%)
Acidobacteria	Acidobacteria	norank	0.12	0.17	0.32	Nitrospirae	Nitrospira	norank	0.21	0.01	0.28
	Holophagae	norank	5.30	5.55	0.00	Planctomycetes	Planctomycetacia	*Gemmata*	0.73	0.68	0.00
	Actinobacteria	uncultured	0.40	0.15	0.46			*Pirellula*	0.96	0.42	1.31
	Thermoleophilia	*Gaiella*	0.30	0.17	0.44			*Planctomyces*	1.81	1.75	0.00
Armatimonadetes	norank	norank	0.07	0.08	0.07			uncultured	0.47	0.20	1.03
Bacteroidetes	Bacteroidia	norank	4.41	1.03	0.71	Proteobacteria	Alphaproteobacteria	norank	0.18	0.14	0.30
	Sphingobacteriia		0.42	0.03	0.00			*Rhodobacter*	0.04	0.02	14.8
	Sphingobacteriia		0.00	0.22	0.48			*Delftia*	0.78	1.47	1.41
Candidate division BRC1	vadinHA17	norank	0.11	0.17	0.03			*Thiobacillus*	1.21	0.01	0.00
	norank	norank	1.94	0.46	0.25		Deltaproteobacteria	*Desulfovibrio*	12.97	6.86	4.30
Chloroflexi	Anaerolineae	*Leptolinea*	0.88	0.12	0.00			norank	21.94	47.44	28.6
	Caldilineae	uncultured	0.98	0.43	0.42		Gammaproteobacteria	*Pseudoxanthomonas*	0.90	0.00	4.63
Firmicutes	Clostridia	*Anaerofustis*	0.01	0.00	0.17	Spirochaetae	Spirochaetes	*Spirochaeta*	0.10	0.05	0.11
		Incertae_Sedis	0.94	0.05	37.88	Synergistetes	Synergistia	uncultured	3.75	0.09	0.02
	Negativicutes	norank	37.26	31.73	0.03	Others			0.81	0.50	1.95

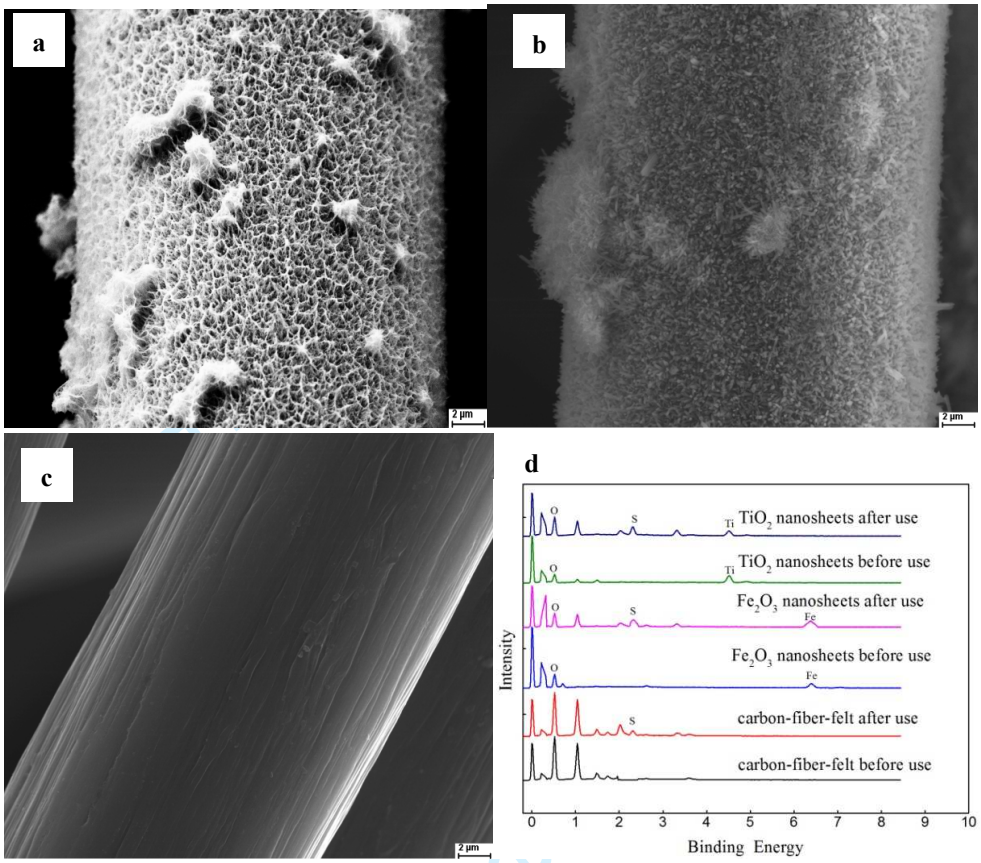

Figure 1

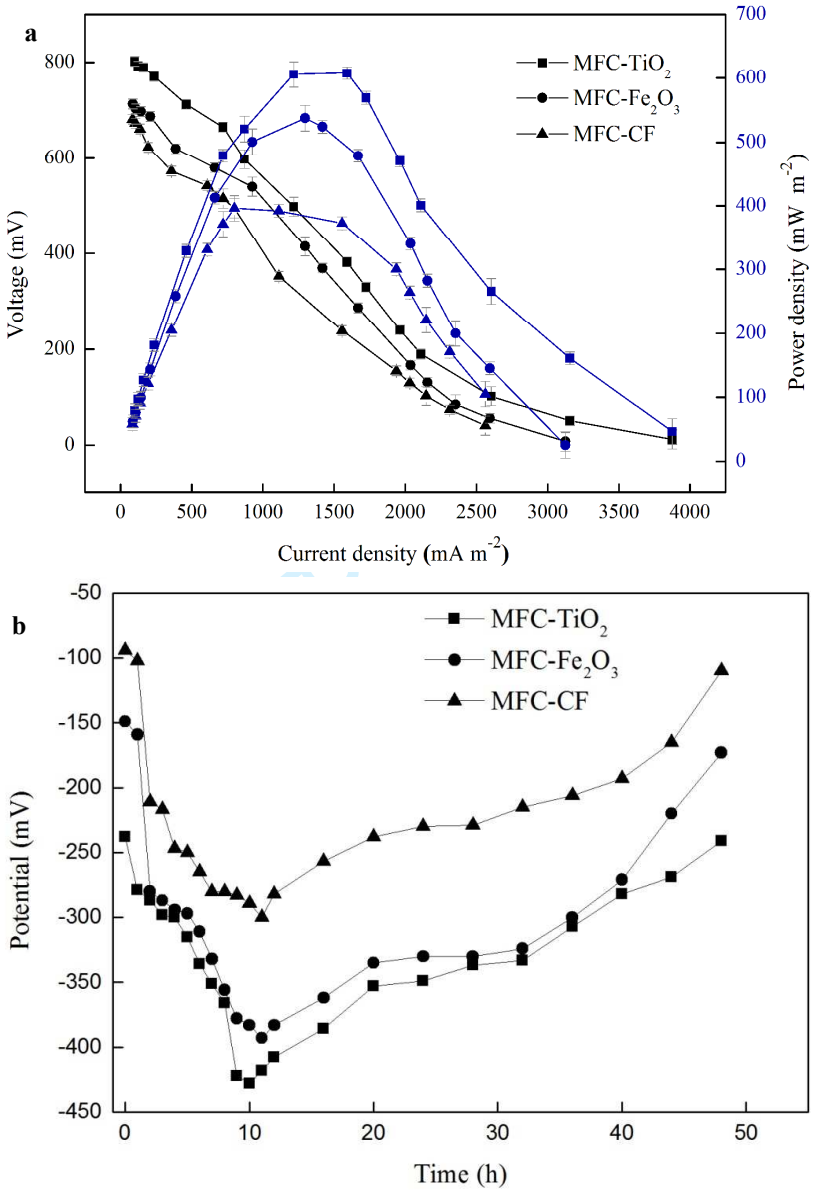
a
MFC-TiO2
MFC-Fe2O3
MFC-CF
Voltage (mV)
Power density (mW m-2)
Current density (mA m-2)
b
MFC-TiO2
MFC-Fe2O3
MFC-CF
Potential (mV)
Time (h)

Figure 2

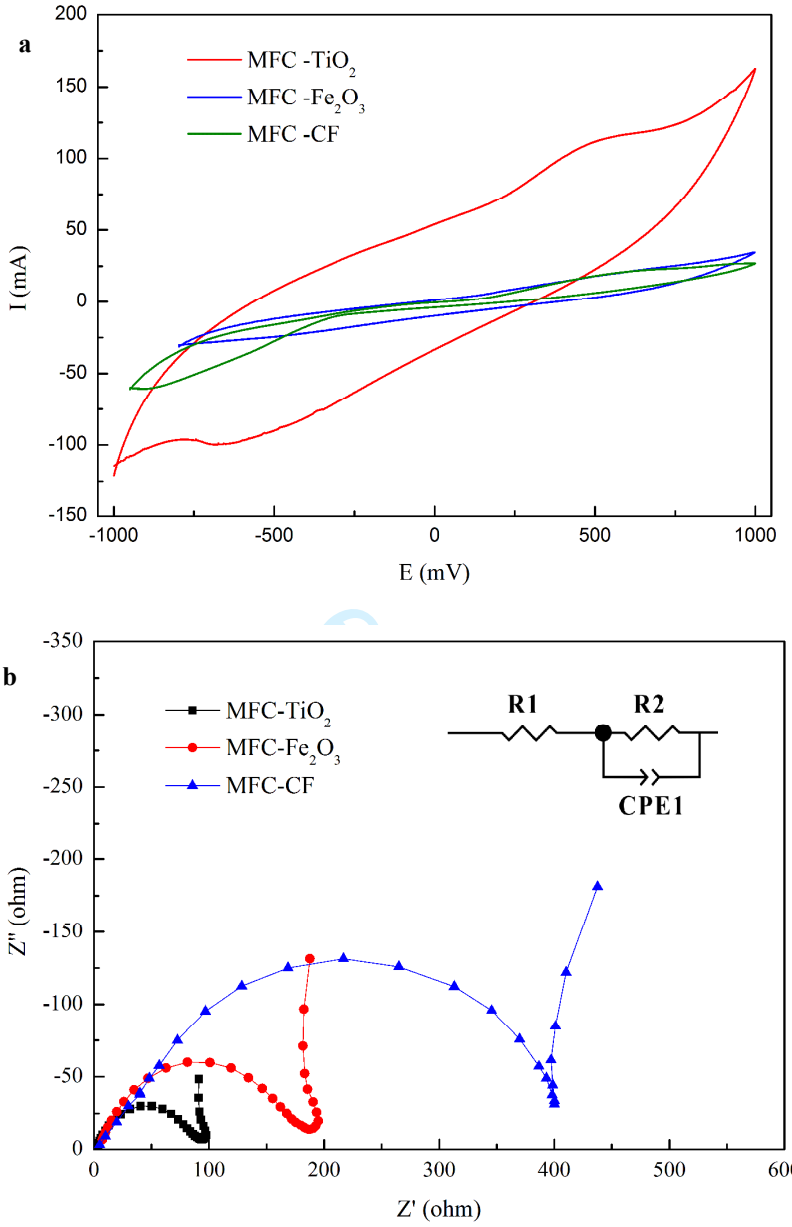
a
MFC -TiO2
MFC -Fe2O3
MFC -CF
I (mA)
E (mV)
200
150
100
50
0
-50
-100
-150
-1000
-500
0
500
1000
b
MFC-TiO2
MFC-Fe2O3
MFC-CF
R1
R2
CPE1
Z'' (ohm)
Z' (ohm)
-350
-300
-250
-200
-150
-100
-50
0
0
100
200
300
400
500
600

Figure 3

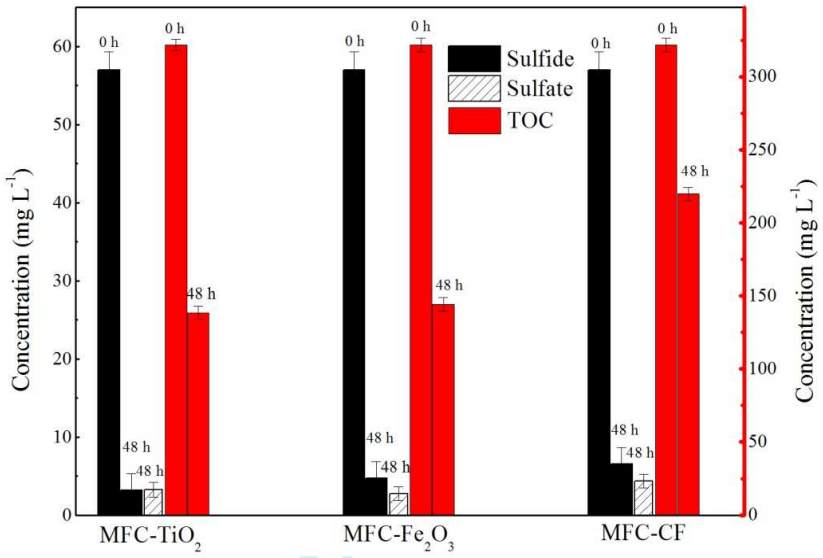

Figure 4

Supporting Information

Enhanced sulfide removal and bioelectricity generation in microbial fuel cells with anodes modified by vertically oriented nanosheets

Meng Yang[a,#], Yuezhi Zhong[a,#], Baogang Zhang[a,*], Jiaxin Shi[a], Xueyang Huang[a], Yi Xing[b,*], Lin Su[c], Huipeng Liu[a], Alistair G.L. Borthwick[d]

[a] *School of Water Resources and Environment, MOE Key Laboratory of Groundwater Circulation and Environmental Evolution, China University of Geosciences (Beijing), Beijing 100083, P. R. China*

[b] *School of Energy and Environmental Engineering, University of Sciences and Technology Beijing, Beijing Key Laboratory of Resource-oriented Treatment of Industrial Pollutants, Beijing 100083, P. R. China*

[c] *State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096, P. R. China*

[d] *School of Engineering, The University of Edinburgh, The King's Buildings, Edinburgh EH9 3JL, UK*

[#] These authors contributed equally to this work.

[*] Corresponding author. Tel.: +86 10 8232 2281; fax: +86 10 8232 1081.

E-mail: zbgcugb@gmail.com, baogangzhang@cugb.edu.cn (B. Zhang); xing_bkd@163.com (Y. Xing).

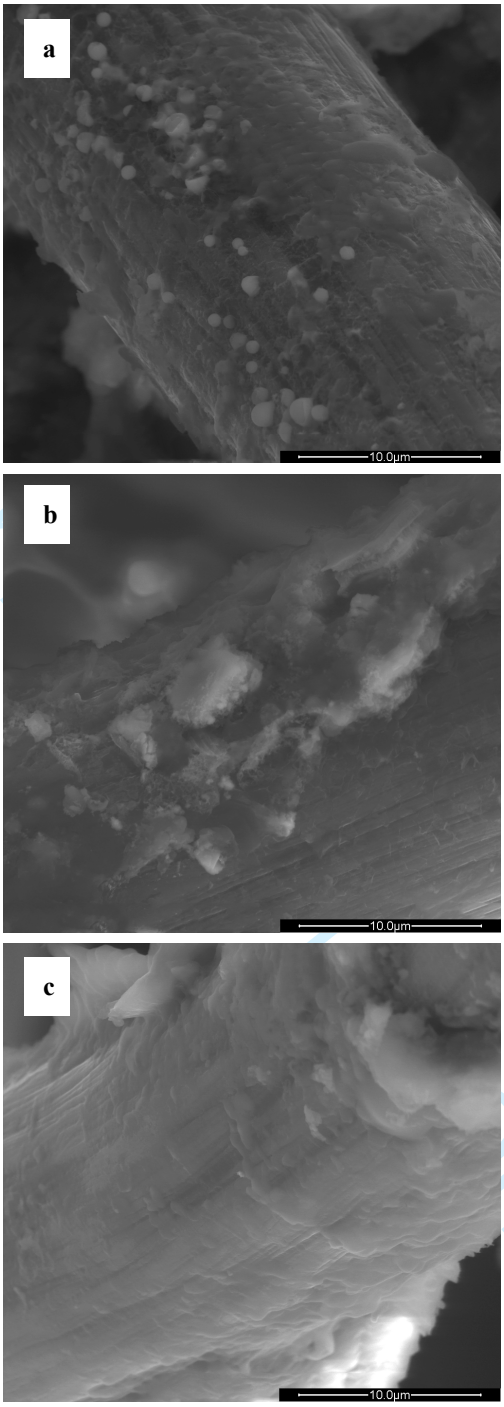

Figure S1. SEM images of anodes in the three MFCs after the whole experiment. (a) MFC-TiO_2; (b) MFC-Fe_2O_3; (c) MFC-CF.